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K. van Frankenhuyzen [1], C. Howard [2], J. Churcher [2], G. Howse [3] and D. Lawrence [3]

Forest Pest Management Institute
Forestry Canada
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7

[1] Forestry Canada, Forest Pest Management Institute

[2] Ontario Ministry of Natural Resources, Pest Management Section

[3] Forestry Canada, Forest Insect and Disease Survey

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TABLE OF CONTENTS

	Page
ABSTRACT	i
RESUME	ii
INTRODUCTION	1
MATERIALS AND METHODS	2
Research Site	2
Application	2
Deposit Assessment	2
Bioassay	4
Egg Mass Survey	5
Defoliation	5
RESULTS AND DISCUSSION	5
ACKNOWLEDGMENT	8
REFERENCES	10

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ABSTRACT

The feasibility of ultra-low volume application of undiluted *Bacillus thuringiensis* formulations for control of the gypsy moth was examined by treating three oak stands with Dipel 8AF at 15 BIU in 0.9 L/ha (undiluted) or at 30 BIU in 1.8 L/ha (undiluted) and 6.0 L/ha (diluted). Branches were collected from upper, middle and lower canopy and assayed for spray deposits as well as toxicity to third-instar gypsy moth larvae. Droplet size distributions in the two undiluted treatments were similar with a number median diameter (NMD) of 49-55 μm and a volume median diameter (VMD) of 117-126 μm. The diluted application resulted in a broader size distribution, with an NMD of 98 μm and a VMD of 180 μm. Droplet density was significantly higher in the 1.8 and 6.0 L/ha treatments (5-10 droplets/cm^2) than in the 0.9 L/ha treatment (3-5 droplets/cm^2). Droplet density decreased significantly from upper to lower canopy in each spray block. Mortality of gypsy moth larvae in the bioassays reached about 50% in the 1.8 and 6.0 L/ha treatments and 30% in the 0.9 L/ha treatment. Regression analysis of mortality versus observed droplet density in the bioassays did not differ between treatments. For both diluted and undiluted applications, a density of 8-10 droplets/cm^2 was required to obtain 50% mortality. All treatments resulted in lower defoliation and egg mass density than in untreated areas, but declining populations confounded the assessment. Application of undiluted formulations containing 16.9 BIU/L in 1.8 L/ha can be recommended for operational control of gypsy moth. The feasibility of using higher potency products in even lower volumes needs to be assessed in future programs.

RÉSUMÉ

La possibilité d'appliquer en très faible volume des formulations non diluées de *Bacillus thuringiensis* pour lutter contre la spongieuse a été examinée : on a traité trois peuplements de chênes avec du Dipel 8AF à 15 UBI par 0,9 L/ha (forme non diluée) ou 30 UBI par 1,8 L/ha (forme non diluée) ainsi que 6,0 L/ha (forme diluée). Des branches ont été prélevées aux niveaux supérieur, intermédiaire et inférieur de la cime; les dépôts laissés par pulvérisation ainsi que la toxicité chez les larves du troisième stade de la spongieuse ont été étudiés. La distribution par taille des gouttelettes était semblable dans les deux traitements avec le produit non dilué, soit avec un diamètre médian en fonction du nombre (DMN) de 49-55 μm et un diamètre médian en fonction du volume (DMV) de 117-126 μm. L'application du produit dilué a eu pour effet d'élargir la distribution des gouttelettes; le DMN était de 98 μm et le DMV était de 180 μm. Il y avait une densité significativement supérieure des gouttelettes lors des traitements à 1,8 et 6,0 L/ha (5-10 gouttelettes/cm^2) que lors du traitement à 0,9 L/ha (3-5 gouttelettes/cm^2). Il y avait une réduction significative de la densité des gouttelettes du niveau supérieur au niveau inférieur de la cime dans chaque parcelle traitée. Lors des essais biologiques, la mortalité des larves de la spongieuse se chiffrait à environ 50 % dans les groupes traités à 1,8 et 6,0 L/ha et atteignait 30 % dans le groupe traité à 0,9 L/ha. Il n'y avait pas de différence entre les différents traitements quant à l'analyse par traitements et quant à l'analyse par régression de la mortalité dans les bioessais par rapport à la densité observée des gouttelettes. Que ce soit sous forme diluée ou non, il fallait une densité de gouttelettes de 8-10 gouttelettes/cm^2 pour atteindre une mortalité de 50 %. Tous les traitements ont atténué la défoliation et diminué la densité des masses d'oeufs, par rapport aux secteurs-témoins, mais les populations généralement faibles de larves n'ont pas aidé à l'évaluation. L'application de formulations sous forme non diluée et contenant 16,9 UBI/L dans 1,8 L/ha peut être recommandée pour les traitements opérationnels de la spongieuse. Il est suggéré de vérifier la possibilité d'utiliser des produits plus actifs et un volume encore moindre lors des futurs programmes.

INTRODUCTION

Bacillus thuringiensis Berliner is widely used for control of the gypsy moth, *Lymantria dispar* L., in eastern North America. Aerial control operations usually involve application of diluted formulations in 6-9.5 L/ha (Grimble and Lewis 1985). Such high volume application rates were used for control of the eastern spruce budworm, *Choristoneura fumiferana*: high-potency formulations became available in the early 1980s (Morris 1984). These formulations contain 12.7 Billion International Units (BIU)/L or more and can be applied undiluted at 30 BIU/ha in 2.4 L or less. Application of undiluted formulations has substantially reduced costs of spruce budworm control operations (Irland and Rumpf 1987), and would have similar benefits in gypsy moth control programs.

Ultra-low volume (ULV) application requires efficient atomization of the spray formulation into small droplets. Micronair rotary atomizers can generate a high proportion of droplets <100 μm in diameter from undiluted high-potency formulations (Yates and Cowden 1986; Van Vliet and Picot 1987). Such droplets impinge effectively on coniferous foliage (Picot et al. 1986) and are efficacious against several defoliators at densities of one droplet per needle or less (Fast et al. 1986; West et al. 1987; Cadogan et al. 1986). However, data are needed on deposition of small droplets in a hardwood canopy and their effectiveness against hardwood defoliators.

A cooperative study was initiated in 1987 to examine droplet deposition and distribution in relation to droplet size in an oak forest canopy following application of diluted and undiluted formulations. The study involved the Forest Pest Management Institute (FPMI) of Forestry Canada, the New Brunswick Research and Productivity Council (RPC) and Abbott Laboratories. The study was continued in 1988 in cooperation with the Pest Management Section of the Ontario Ministry of Natural Resources (OMNR) and the Forest Insect and Disease Survey (FIDS) of Forestry Canada. Specific objectives were: (1) to examine droplet deposition in an oak canopy in relation to application volume under operational spraying conditions; (2) to relate observed spray deposits to gypsy moth mortality in bioassays of sprayed foliage; (3) to assess efficacy of diluted and undiluted treatments in terms of foliage protection and egg mass reduction; and (4) to examine feasibility of undiluted application at half the recommended application rate. These objectives were addressed by treating six blocks at 30 or 15 BIU/ha in 0.9 L (three replicates), 1.8 L (two replicates) or 6.0 L (one replicate). The Pest Management Section was in charge of spray application. Spray deposition was assessed by RPC in each spray block and is the subject of a separate report. Biological assessment was conducted by FIDS and FPMI in the three blocks that had suitable gypsy moth populations, one in each treatment, and those results are reported here.

MATERIALS AND METHODS

Research Site

The experiment was conducted in May 1988 in Lavant Township in southeastern Ontario (Fig. 1). The selected blocks (50 -230 ha) contained predominantly red oak (*Quercus rubra* L., 60-90%) and red maple (*Acer rubrum* L., 10-40%), with a light mix of white birch (*Betula papyrifera* Marsh.), poplar (*Populus balsamifera* L.), balsam fir (*Abies balsamea* L.), and sugar maple (*A. saccharum* Marsh.). Dominant trees were generally 15-20 m in height. In each block a total of 15 oak trees were selected at 20-40 m intervals in a line perpendicular to flight direction of the spray aircraft. Crown height of the sample trees varied between 12 and 18 m.

Gypsy moth infestation in the three blocks was first detected in 1986. Populations in block A and B were high enough (>1200 egg masses/ha) to warrant aerial treatment with *B. thuringiensis* in 1987. Block B was scheduled for treatment in 1988 as well and OMNR agreed to allocate half of the 700 ha spray block, with appropriate buffer zones, to our experimental program. Block C formed a natural extension of the infestation in block B.

Application

All blocks were treated with Dipel 8AF (ABG 6167), which is an aqueous flowable formulation produced by Abbott Laboratories (Chicago, Ill.) that contains 16.9 BIU/L. To facilitate deposit assessment, Erio acid red dye XGN250 (St. Lawrence Aniline, Brockville, Ont) was added as a tracer at 0.4% (weight:volume). The mixture was applied by a Piper Brave fitted with four Micronair AU4000 atomizers. The aircraft flew 15-25 m above the canopy at 180 km/h using 60-m lane separations and was guided by a Cessna 172 pointer aircraft. Both planes operated from a private airstrip at Westport. Dipel was applied undiluted in 1.8 L/ha (block A) and 0.9 L/ha (block B) or diluted with water in a 1:2.33 ratio in a total volume of 6.0 L/ha (block C). Sprays were applied under standard operational conditions in the early morning (Table 1).

Deposit assessment

Distribution of spray deposits in the canopy was examined by sampling at three canopy levels. The crown of each sample tree was visually divided into an upper, middle and lower level. Starting one hour after application, one branch was collected from each level with a pole pruner and five twigs were taken from each branch. Cut ends of the twigs were wrapped in wet cotton and aluminum foil. Twigs were then placed individually in plastic boxes and transported in coolers to a field laboratory at the airstrip for assessment of deposits and biologi-

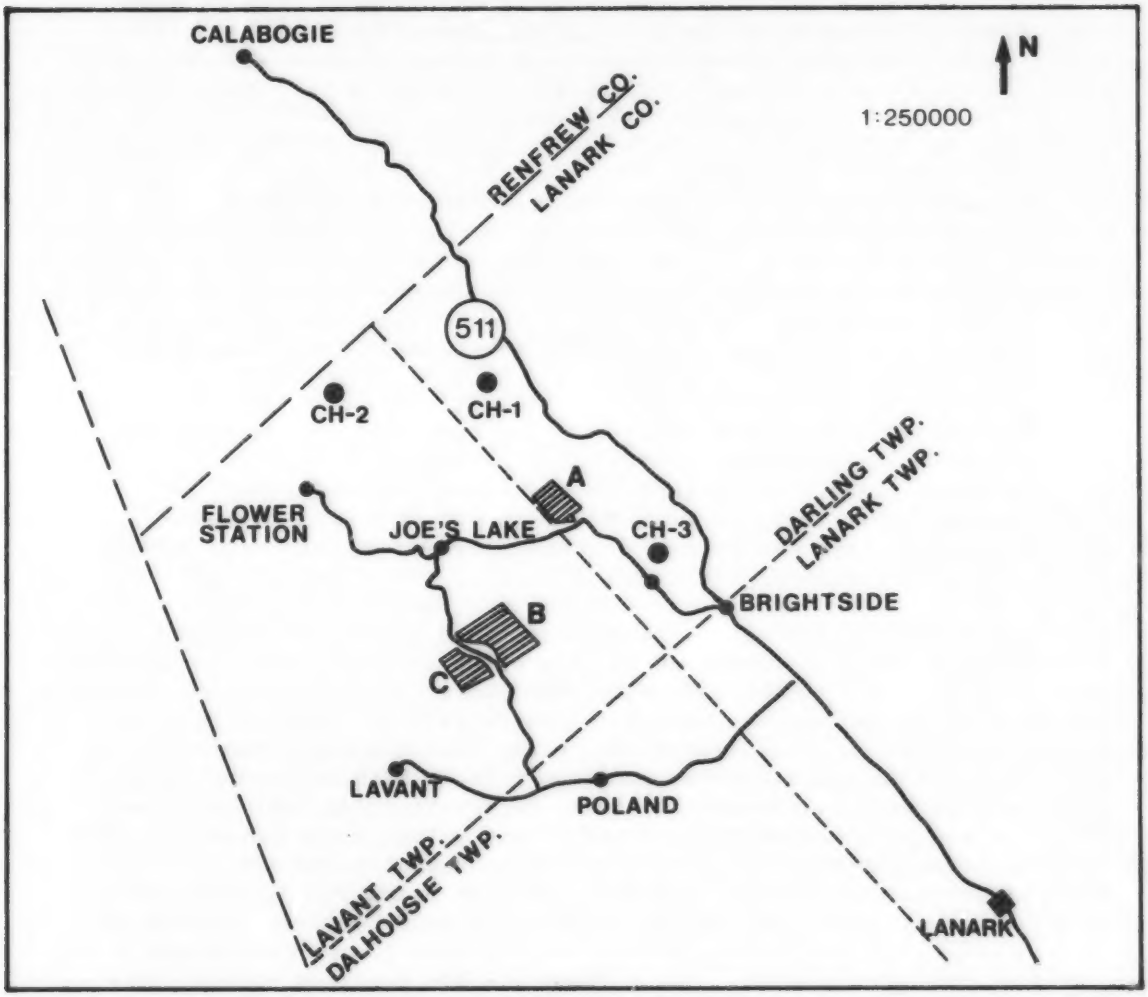

Figure 1. Location of the spray blocks (A, B and C) and untreated checks (1: Napier Hill; 2: Clyde Lake Mountain; 3: French Line) in southeastern Ontario.

cal activity. Spray deposits were estimated by examining one leaf from each twig under the microscope within 5 h of collection. The number of droplets were counted in five randomly selected fields of view for a total of 1.6 cm^2 per leaf surface. Droplet size was estimated by measuring 500 droplets in each treatment, using a Wild Leitz digital length measuring unit (Wild Heerbrug, Willowdale, Ont).

Table 1. Summary of application parameters

	Block A	Block B	Block C
Date of treatment	May 26	May 28	May 27
Size of block (ha)	70	230	50
Foliage expansion	75%	80%	60%
Instar development:			
% first instar	11	10	10
% second instar	46	74	74
% third instar	43	16	16
Time of application	0630-0645	0615-0645	0600-0645
Volume rate (L/ha)	1.8	0.9	6.0
Dosage rate (BIU/ha)	30	15	30
Micronair blade angle	35	35	35
Emission rate (L/min)	33.3	16.6	111
Pressure (psi)	29	38	26
Variable Restrictor #	11	7	bypass

Droplet density estimates were combined for the 15 sample trees in each spray block to obtain an average value from 75 leaves per canopy level. Variation in droplet density among spray blocks and canopy levels was examined by two way analysis of variance (ANOVA). Droplet densities were subjected to log transformation to approach a normal probability distribution. Pairwise, pooled variance t-tests with Bonferroni probabilities were used to compare treatment means within each block (Dixon 1983). The number median diameter (NMD) and volume median diameter (VMD) for each treatment were calculated according to Johnstone (1978). Spray deposits in the undiluted treatments consisted primarily of spherical or hemisperical droplets, indicating complete or near-complete evaporation. Spray deposits in the diluted treatment more than 100 μm in diameter consisted mostly of stains. Stain diameters are reported without conversion to actual droplet diameter.

Bioassay

Biological activity of spray deposits was examined by bioassay of the twigs collected from the three canopy levels. Each twig was placed in a ventilated plastic cage (18 x 8.5 x 4.5 cm) with its cut end in

water and received five early-to-mid third-instar larvae. Larvae came from egg masses that were collected in the fall of the previous year at Rice Lake (Peterborough, Ont). Cages were kept outside, sheltered from rain and direct sunlight. Larvae were transferred to unsprayed foliage on the fourth day and examined for mortality thereafter at 3-4 day intervals until cumulative mortality had stabilized (15-17 days). Natural mortality in cages was determined on 10 twigs collected from unsprayed trees on each treatment day. Larval mortality at each canopy level was related to observed droplet density for three groups of 5 sample trees each (n=15).

Egg mass survey

Egg mass densities were assessed in April and October, using 8 or 9 randomly selected modified Kaladar plots (MKP). These plots consist of 10 x 10 m squares. Egg masses were counted on all tree boles within this area and on the ground in 10 subplots of 1 x 1 m. The reduction in egg mass density was compared with the reduction observed by FIDS in three nearby untreated check blocks (Fig. 1).

Defoliation

Defoliation estimates were conducted by FIDS in late July. One 45-cm branch was collected from the midcrown of 10 trees in each of the MKP's. Defoliation was estimated by scoring the degree of defoliation of each leaf on the branch. Defoliation in the treated blocks was compared with estimates obtained from the three control blocks.

RESULTS AND DISCUSSION

The overall objective of this study was to assess the feasibility of applying undiluted *B. thuringiensis* for gypsy moth control. In 1987 we concluded that ULV application results in more uniform distribution of spray deposits within the canopy, due to better canopy penetration by smaller droplets, and equivalent mortality of gypsy moth larvae when compared to application of diluted product at higher volumes (van Frankenhuyzen et al. 1989). Results of the 1988 study generally support these observations.

The deposit data reported here are based on only one spray block per treatment. Estimates for the other blocks will be reported by RPC at a later date. Preliminary observations based on our data are reported here.

Mean droplet densities were significantly affected by spray treatment and position in the canopy (Table 2). Application of 6.0 and 1.8 L/ha resulted in comparable droplet densities, which were about 2-

fold higher than in the 0.9 L/ha treatment (Fig. 2). Distribution of spray deposits varied significantly within the tree canopy (Table 2). None of the treatments resulted in a uniform distribution of spray deposits throughout the canopy; droplet densities in the upper canopy were always significantly higher than in the lower canopy (Fig. 2). However, the difference between upper and middle canopy was largest in the 6.0 L/ha treatment and was not significant in the two undiluted treatments, despite more advanced expansion of foliage at the time of treatment, suggesting that undiluted application may have resulted in better canopy penetration.

Table 2. Analysis of variance for droplet density on upper and lower leaf surface combined

Source of variation	df	*F*	*P*-value
spray treatment	2	33.3	0.000
canopy level	2	18.0	0.000
spray x canopy	4	1.9	0.102
error	640		

Droplet size distributions were similar in the two undiluted sprays. More than 85% of all droplets were less than 100 μm in diameter (Fig. 3, blocks A and B), resulting in an NMD of 49-55 and a VMD of 117-126 μm. Size distribution in the 6.0 L/ha treatment was broader, with about 55% of all droplets under 100 μm and a larger NMD (98 μm) and VMD (188 μm) (Fig. 3, block C).

Spray deposits in the 1.8 and 6.0 L/ha treatments resulted in comparable mortality of third-instar gypsy moth larvae in the bioassays (respectively 46.1±2.4% and 51.0±3.3%, block average ± SE, n=45). Lower spray deposits in the 0.9 L/ha treatment resulted in significantly lower mortality (30.4±3.2%). Larval mortality on unsprayed foliage never exceeded 5%. Mortality decreased from upper to lower canopy similar to the observed distribution of spray deposits (Fig. 2). There was a linear relationship between mean mortality and mean droplet density on the twigs in each treatment. The relationship was weakest in block C. Regression lines did not differ significantly in either slopes or intercepts (ANOVA of regression coefficients: P=0.213) and were adequately described by a common line (Table 3). A density of 8-10 droplets/cm^2 was the estimated requirement for 50% mortality. In the 1987 study we obtained a similar estimate for Dipel 8AF applied at 1.8 L/ha but a much higher LD_{50} for application of diluted product (van Frankenhuyzen et al. 1989). The reason for this difference is not readily apparent.

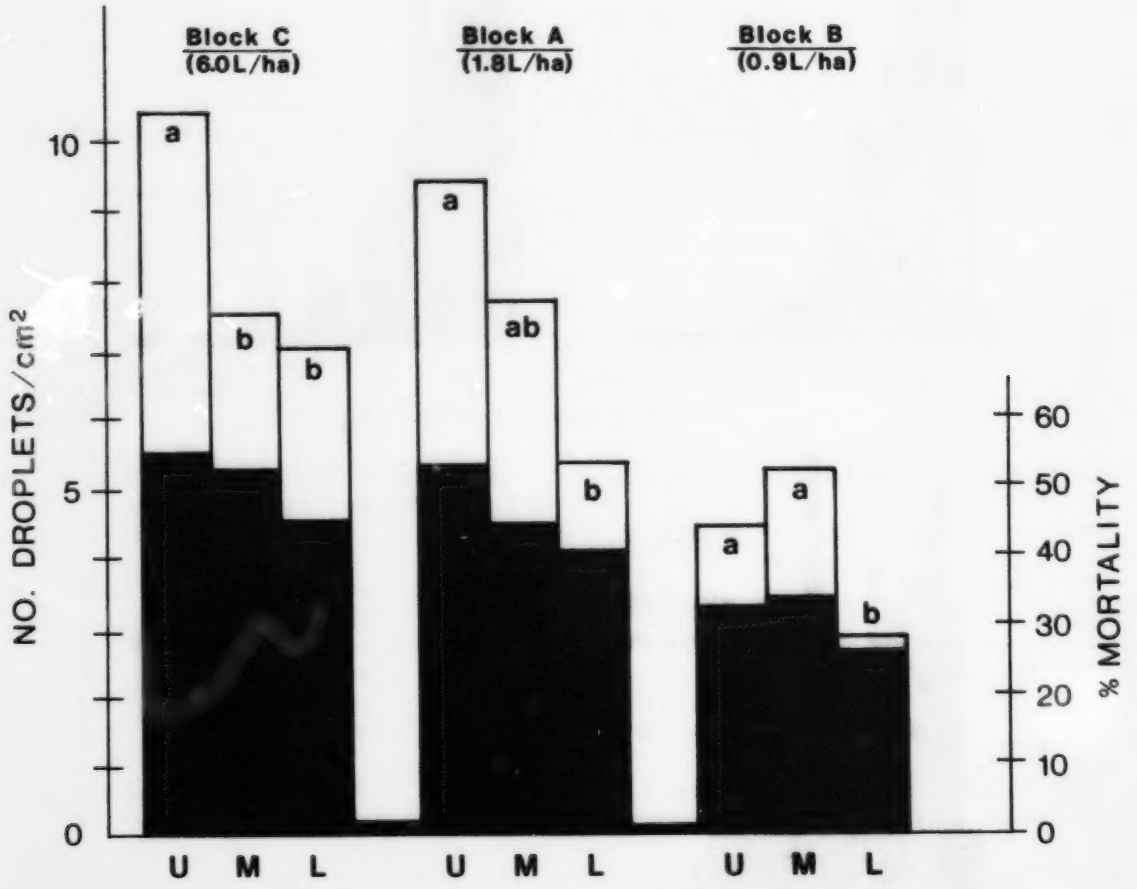

Figure 2. Mean number of spray droplets per cm² (open bars) and resulting mortality of third-instar gypsy moth larvae (solid bars) in bioassays of foliage collected from the upper (U), middle (M) and lower (L) canopy of an oak forest following treatment with Dipel 8AF at 6.0, 1.8 or 0.9 L/ha. Different letters within each spray block indicate significantly different means ($P<0.05$, Bonferroni).

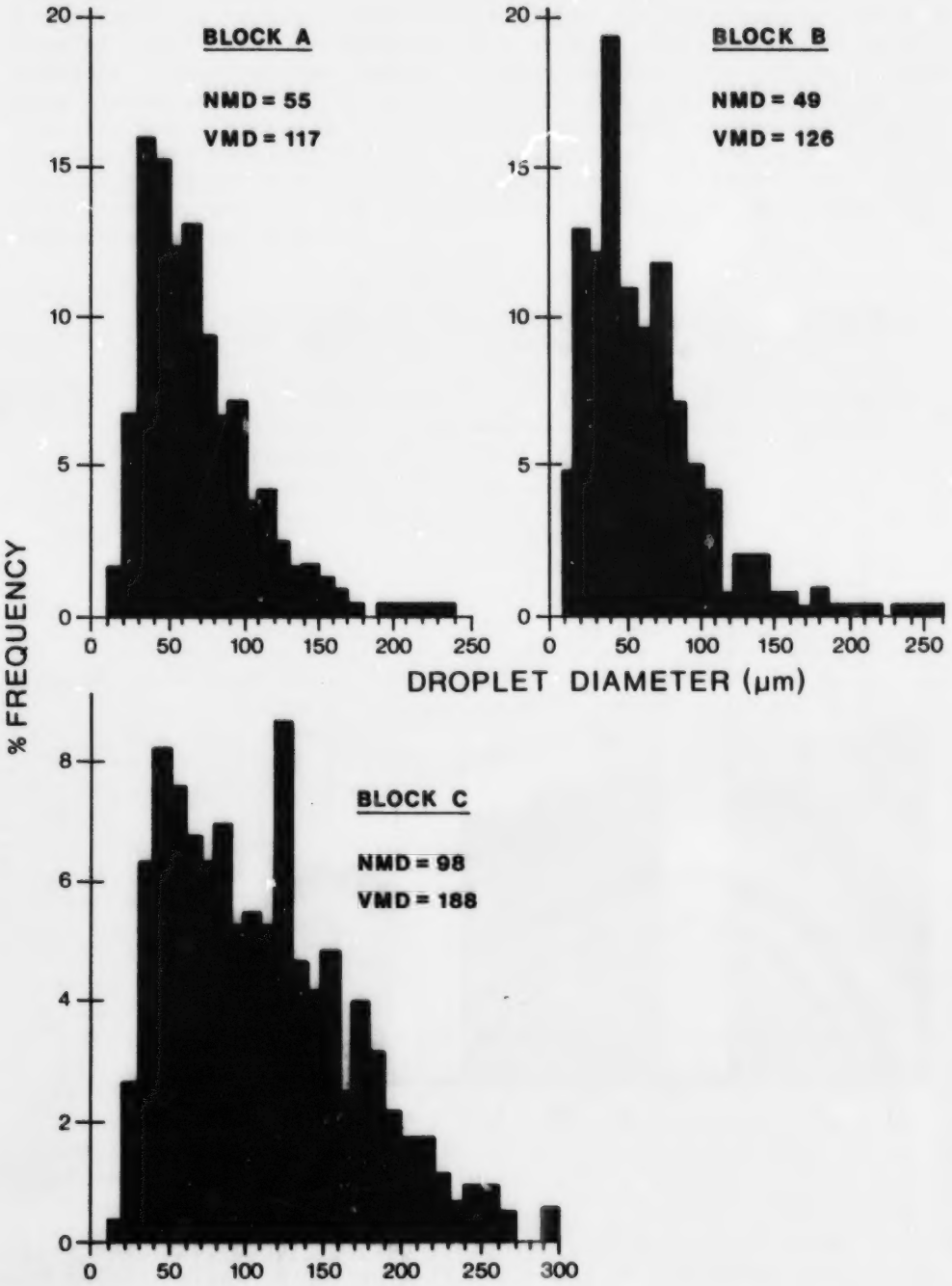

Figure 3. Frequency distribution of droplet sizes observed on foliage after application of 1.8 L/ha (block A), 0.9 L/ha (block B) or 6.0 L/ha (block C).

Table 3. Regression of % mortality of third-instar gypsy moth larvae against droplet density in the bioassays

Block	Treatment L/ha	Regression equation	R^2	*P*	Estimated LD_{50} (droplets/cm^2)
A	1.8	Y = 22.3 + 3.16 X	71.8	0.004	8.8
B	0.9	Y = 16.0 + 3.40 X	53.4	0.025	10.0
C	6.0	Y = 33.8 + 2.08 X	30.8	0.121	7.8
Common		Y = 18.7 + 3.55 X	0.67	0.001	8.8

Larval populations expected on the basis of the spring egg mass counts (Table 4) did not materialize because many egg masses failed to hatch. Low larval populations resulted in low defoliation in the check blocks, ranging from 15-57% with an average of 28% (Table 4). Egg mass densities in the check blocks were reduced by 25-98%, with an average reduction of 75% (Table 4). In the treated blocks, defoliation was consistently lower (12-16%) and egg mass reduction slightly greater (80-90%) than in the untreated areas, indicating that each treatment had a measurable effect. The relative effectiveness of the various treatments, however, remains equivocal because of the low population densities encountered, warranting further studies to confirm our results.

Table 4. Mean defoliation (%) and change in mean egg mass density in treated and untreated blocks

Block	Treatment L/ha	N	% Defoliation (±SE)	No. egg masses/ha (±SE) Spring	Fall	% Change
A	1.8	9	12.3 ± 0.8	3100 ± 664	455 ± 172	85
B	0.9	9	14.7 ± 0.6	1488 ± 340	266 ± 104	82
C	6.0	8	15.6 ± 0.9	2587 ± 505	250 ± 47	90
Check		6	27.8 ± 6.3	6583 ± 1288	1600 ± 698	75

This study demonstrates once again that ULV application of undiluted formulations can result in spray deposits and larval mortality equivalent to application of diluted product in higher volumes. Based on our findings, we recommend application of undiluted formulations containing 16.9 BIU/L at 30 BIU in 1.8 L/ha for operational control of gypsy moth. However, whether such low spray volumes are effective against healthy, increasing populations remains to be determined. The use of lower spray volumes also merits further investigation. Although applica-

tion of 0.9 L/ha in this study resulted in significantly lower spray deposits and less larval mortality, the use of more concentrated formulations may improve results by increasing the effectiveness of low spray deposits. In future programs we intend to assess the effectiveness of highly concentrated formulations that are already available and which can be applied at 30 BIU in 0.9 L/ha or less.

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